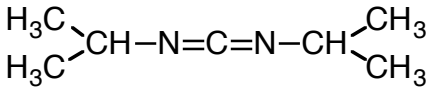
NTP
National Toxicology Program

# Toxicology and Carcinogenesis Studies of Diisopropylcarbodiimide in F344 Rats and B6C3F$_1$ Mice by Dermal Route of Exposure



Diisopropylcarbodiimide

NTP
National Toxicology Program

# Study Rationale

- Diisopropylcarbodiimide (DIC) and Dicyclohexylcarbodiimide (DCC) were nominated by the NCI for toxicity and carcinogenicity studies as representatives of the carbodiimide chemical class
- DIC and DCC are used as stabilizing, coupling and condensing agents
- The potential for exposure exists during the synthesis of polypeptides and other chemicals in the chemical and pharmaceutical industries, as well as during protein synthesis in the recombinant DNA industry
- Results of DCC studies will be reported later

NTP
National Toxicology Program

# Studies Performed by the NTP

- 2-Week, 13-Week and 2-Year studies in F344 rats and B6C3F$_1$ mice by dermal route of exposure
- Clinical pathology
- Reproductive tissue evaluations and estrous cycle characterization
- Absorption and distribution studies in rats and mice
- Genetic toxicity studies
- Studies in genetically modified mouse models

NTP
National Toxicology Program

# 3-Month Study Results in Rats

- DIC dose levels administered were 0, 10, 20, 40, 80, or 160 mg/kg
- Top two groups died or were sacrificed in moribund state
- Significant body weight decreases in 40 mg dose group
- Clinical observations included irritation at the site of application, seizures, ataxia, and abnormal breathing
- Significantly increased skin lesions (hyperplasia and inflammation) at the site of application and non-neoplastic lesions in brain, lung and liver of 80 or 160 mg dose groups
- No changes in reproductive tissue evaluations or clinical pathology
- Slight dermal absorption (1-2%)

NTP
National Toxicology Program

# Results of 3-Month Rat Studies (contd.)
# Major Non-neoplastic Lesions

| DIC (mg/Kg) | 0 | 10 | 20 | 40 | 80 | 160 |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Skin-hyperplasia (SOA) | 0 | 5* | 7** | 10** | 10** | 3 |
| necrosis, focal | 0 | 0 | 0 | 0 | 0 | 9 |
| inflammation, chronic | 0 | 0 | 0 | 1 | 7** | 10** |
| Brain- edema, focal | 0 | 0 | 0 | 0 | 5** | 1 |
| hemorrhage | 0 | 0 | 0 | 0 | 1 | 4** |
| necrosis, focal | 0 | 0 | 0 | 0 | 8** | 0 |
| **Female** | | | | | | |
| Skin-hyperplasia (SOA) | 1 | 2 | 3 | 5* | 10** | 10** |
| necrosis, focal | 0 | 0 | 0 | 0 | 0 | 10** |
| inflammation, chronic | 0 | 0 | 0 | 0 | 7** | 10** |
| Brain- edema, focal | 0 | 0 | 0 | 0 | 5** | 2* |
| hemorrhage | 0 | 0 | 0 | 0 | 5** | 6** |
| necrosis, focal | 0 | 0 | 0 | 0 | 8** | 2* |

N=10, *p<0.05, **p<0.01

NTP
National Toxicology Program

# 2-Year Study Results in Rats

- DIC dose levels were 0, 10, 20 or 40 mg/kg to males and females
- Survival of 20 mg/kg was significantly greater than that of the vehicle controls, other groups were comparable
- Body weights of 40 mg/kg male and female were generally lower than controls
- Clinical findings frequently observed in 40 mg/kg male rats included ataxia, excitability, impaired gait, low muscle tone, abnormal breathing, lethargy, vocalization and clonic seizures
- No neoplastic lesions related to DIC treatment

# 2-Year Study Results in Rats (contd.)
# Non-neoplastic lesions

## Males

**Brain- hemorrhage (1, 0, 1, 15$^{**}$ )**
**necrosis, neuron (0, 1, 0, 16$^{**}$)**
**arteriole, necrosis, fibrinoid (0, 0, 0, 5$^{*}$)**
**Lung- hemorrhage (6, 6, 7, 17$^{**}$)**
**Skin- epidermal hyperplasia (1, 10$^{**}$, 29$^{**}$,19$^{**}$)**
**inflammation chronic (0, 6$^{*}$, 12$^{**}$, 11$^{**}$)**
**Eye- cornea, hyperplasia (0, 0, 1, 5$^{*}$)**
**cornea, inflammation (0, 1, 5$^{*}$, 23$^{**}$)**

## Females

**Skin- epidermal hyperplasia (1, 5, 16$^{**}$, 21$^{**}$)**
**inflammation, chronic (0, 0, 3, 10$^{**}$)**
**Lung- inflammation, chronic (10, 22$^{**}$, 19$^{*}$, 10)**
**alveolar epithelium, hyperplasia (3, 4, 10$^{**}$, 1)**

NTP
National Toxicology Program

# 3-Month Study Results in Mice

- DIC dose levels used were 0, 17.5, 35, 70, 140 or 280 mg/kg in ethanol
- No survival at the top dose level, only one male and female survived at the next dose group
- Clinical observations in top two dose levels included ataxia, comatose conditions, convulsions, irritation at the site of application
- No major effects on hematology or reproductive parameters
- Dose related increases in epidermal hyperplasia and inflammation at the site of application up to 70 mg group and thymus atrophy at 140 and 280 mg group

NTP
National Toxicology Program

# 2-Year Study Results in Mice

- Dose levels used were 0, 10, 20 or 40 mg/kg
- Survival and body weights of all dose groups was similar to that of control groups
- No clinical findings related to DIC treatment
- In male mice at the site of application epidermal hyperplasia (2/50, 3/50, 10/50*, 1/50) and focal inflammation (2/50, 2/50, 9/50*, 1/50) were observed
- There were no neoplastic lesions observed

NTP
National Toxicology Program

# Genetic Toxicity

- Not mutagenic in *Salmonella* tests
- *In vivo,* the frequency of micronucleated normochromatic erythrocytes was significantly increased in 3-month male and female mouse studies
- Significant increases in micronucleated reticulocytes and normochromatic erythrocytes were seen in an additional 4-month dermal study
- In general, no significant increases in micronucleated reticulocytes were seen in acute IP exposure studies in rats or mice

NTP
National Toxicology Program

# Conclusions

- *No evidence of carcinogenic activity* **of diisopropylcarbodiimide in male or female F344 rats or B6C3F$_1$ mice.**

- **Neurotoxicity associated with Diisopropylcarbodiimide administration in male rats.**

NTP
National Toxicology Program

# NTP Technical Reports Review Subcommittee Meeting

## Diisopropylcarbodiimide

## TR 523